

Transfection of SF9 best procedure

- 1- Start from suspension cells (detaching the cells from adherent flasks may cause cell disruption).
- 2- Count 8×10^5 cells per well and plate them in adherent media (centrifuge them and resuspend them in this media).
- 3- During centrifugation prepare plating media: 1.5 ml unsupplemented media +10% FBS and 8.5 ml of unsupplemented media.
- 4- After 15 minutes the cells should be attached. Replace the media with the plating media.
- 5- While waiting for cells to attach prepare the mixes:

Mix A

Cellfectin 8ul+

Grace's media unsupplemented 100ul.

Mix B

LR recombination product 10ul

Grace's media unsupplemented 100ul

Add B to A and wait for 30 minutes.

Add to the cells dropwise.

The day after replace the media with supplemented +FBS 10%+ Ab/Am+ ganciclovir. Wait at least 72 hours and proceed with testing the cells by western blot.